JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 1994, p. 2559–2563
0095-1137/94/$04.00+0

Vol. 32, No. 10

Multicenter Evaluation of a Fully Automated Screening Test, VIDAS HIV 1+2, for Antibodies to Human Immunodeficiency Virus Types 1 and 2

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Received 25 March 1994/Returned for modification 16 June 1994/Accepted 28 July 1994

A multicenter study was done to evaluate the sensitivity, specificity, and efficiency of a new screening test for the simultaneous detection of human immunodeficiency virus type 1 and 2 (HIV-1 and HIV-2) antibodies. The VIDAS HIV 1+2 (bioMérieux, Marcy l'Etoile, France) is a fully automated enzyme-linked fluorescent immunoassay that uses synthetic peptides from immunodominant regions of gp41 of HIV-1 and gp36 of HIV-2 as antigens. A total of 2,984 samples were evaluated with this system in six different laboratories, and the results were compared to those obtained with other enzyme-linked immunosorbent assays. The VIDAS HIV 1+2 assay showed a very good performance in terms of sensitivity (100%) and specificity (99.6%), requiring minimal manipulation and short incubation time (32 min) to give results similar to or better than those of the other enzyme-linked immunosorbent assays used for screening.

The circulating antibodies produced in response to human immunodeficiency virus type 1 and type 2 (HIV-1 and HIV-2) proteins form the basis for the current screening tests that detect the presence of HIV infection. The enzyme-linked immunosorbent assay (ELISA) is the most widely used serological test for HIV-1 and HIV-2 antibody detection (4, 15, 20).

An important feature of the immune response to HIV is that specific antibodies reach detectable amounts only 9 to 12 weeks after acute infection. Therefore, recent HIV infections will not be detected by antibody tests prior to almost complete seroconversion, and therefore recently infected individuals could be considered incorrectly to be noninfected persons. This is extremely important when screening blood samples from low-risk populations and particularly blood donors. An important tool to minimize this risk is to improve the quality of screening tests, using more sensitive ELISAs, combined with an almost 100% specificity.

To increase the specificity of ELISA tests, recombinant protein (RP)- and synthetic peptide (SP)-based systems have been developed and evaluated (1–3, 6, 8, 10–14, 16, 17). Here we report the results of a multicenter study conducted to assess the sensitivity and specificity of a new fully automated ELISA-based system (VIDAS HIV 1+2 [referred to as VIDAS]). Evaluations were performed in six different laboratories, using the VIDAS assay; the results were compared with those from other ELISAs and Western blot (WB) (immunoblot) assays.

MATERIALS AND METHODS

VIDAS. The VIDAS assay (bioMérieux, Marcy l'Etoile, France) is an enzyme-linked fluorescent assay that detects HIV-1 and HIV-2 antibodies simultaneously, and it was performed according to the manufacturer's instructions. Briefly, the instrument uses a disposable pipette tip called the solid-phase receptacle (SPR), coated with HIV antigens, that also acts as a pipetting device. All the ready-to-use reagents are contained in a sealed strip. The specimen (serum or plasma) is added to the reagent strip (0.1 ml), and all the next sequential steps of the test are done automatically, without any further manipulation. The total time needed to complete the reaction is 32 min. VIDAS uses an assay principle that combines ELISA with a final reading using blue fluorescence. HIV antibodies will be detected, after binding to antigen (in SPR), by the addition of anti-human immunoglobulin G conjugated with alkaline phosphatase. The substrate, which will give the soluble fluorescent product, is 4-methylumbelliferyl-phosphate. The intensity of the fluorescence is measured by an optical scanner in VIDAS, and the results are expressed in relative fluorescence values (RFV). The HIV antigens used in the SPR are synthetic peptides, corresponding to the immunodominant regions of the transmembrane glycoproteins of HIV-1 and HIV-2.

Sera. A total of 2,984 samples were studied in 6 different laboratories (see Table 1 for details). A total of 948 positive samples were evaluated (Table 1), including 222 anti-HIV-2 positive samples and 8 serum specimens with reactivity to both HIV-1 and HIV-2. We also analyzed five samples from two individuals who seroconverted to positive anti-HIV-1 immune response. The criteria to discriminate the positive and negative samples were always the same, regardless of where the evaluation was carried out: each sample included in the study was tested twice (in different runs) with the same ELISA (validation and interpretation of the results were done according to manufacturers' instructions); if the two results were negative, the sample was considered negative; if at least one of the two ELISAs was positive, the sample was further analyzed by WB

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TABLE 1. Distribution and details of 2,984 samples studied in six laboratories

Type of serum sample[a]	No. of samples from laboratory						
	Italy (SUC)	Spain (BHB)	Portugal (FP)[b]	France			Total
				BH	DH	RBTC	
Positive samples							
Anti-HIV-1 positive samples	184	133	50	301	19	17	704
Anti-HIV-2 positive samples			146	65	10	1	222
Anti-HIV-1 and HIV-2 samples			8				8
BBI PRB102 (anti-HIV-1 low-titer panel)				14			14
Total of positive samples	184	133	204	380	29	18	948
HIV-1 seroconversions[c]				2			2
Negative samples							
Negative at random	164	101	249	104		1,081	1,699
RF^+		15					15
EBV^+		9					9
African					97		97
Pregnant women					50		50
Hemodialysis patients					50		50
Leprosy patients					47		47
Syphilis patients		3					3
HBs Ag^+		7					7
Anti-*gag*		36			18		54
Total of negative samples	164	171	249	104	262	1,081	2,031
Total of samples studied	348	304	453	489	291	1,099	2,984

[a] RF^+, rheumatoid factor positive; EBV^+, Epstein-Barr virus positive; HBs Ag^+, hepatitis B virus surface antigen positive.
[b] FP, Faculty of Pharmacy, University of Lisbon; other laboratories are defined in the text.
[c] Two panels with a total of five samples.

for HIV-1 and/or HIV-2. The interpretation of WB results was done according to World Health Organization criteria (19).

In the negative group (n = 2,031) we included 332 "difficult negatives" (Table 2), i.e., samples that frequently gave false-positive results in other ELISAs, particularly in those based on viral lysate (VL) ELISA procedures (7, 9). In this group, 54 samples (16.3%) were repeatedly reactive only to *gag* proteins of HIV-1 (p24 or p18) in WB analysis. In addition, these "anti-*gag*" samples were positive in first-generation ELISAs, and they were always negative to HIV p24 antigen (p24 Ag). All of them were from low-risk patients, without any clinical sign of HIV infection; they were monitored for at least 1 year, and none of them seroconverted, showing always the same WB reactivity. Only the samples with these conditions were included in this group.

TABLE 2. Specificity study with difficult negative samples

Type[a]	No. of samples	No. of positive results	
		VIDAS HIV 1+2	Abbott Recombinant HIV 1/HIV 2 EIA
RF^+	15	0	0
EBV^+	9	0	0
African	97	2	0
Pregnant women	50	0	0
Hemodialysis patients	50	0	0
Leprosy patients	47	1	1
Syphilis patients	3	0	1
HBs Ag^+	7	0	0
Anti-*gag*	54	2	6
Total	332	5	8
Specificity		98.5%	97.6%

[a] RF^+, rheumatoid factor positive; EBV^+, Epstein-Barr virus positive; HBs Ag^+, hepatitis B virus surface antigen positive.

Finally, we also analyzed an anti-HIV-1 Low Titer Performance Panel (lot no. PRB102) from Boston Biomedica Inc.; this panel includes only positive samples (n = 14) with a signal cutoff of <4 on at least three Food and Drug Administration-licensed tests and a positive WB or an indeterminate blot when a subsequent sample from the same donor was confirmed WB positive.

ELISAs. The VIDAS assay was compared with the following ELISAs: Abbott recombinant HIV-1 EIA and Abbott recombinant HIV-1 and HIV-2 EIA (Abbott Laboratories, North Chicago, Ill.); Enzygnost HIV 1+2 and Enzygnost anti-HIV micro (Behring, Behringwerke Diagnostica, Germany); ELAVIA I, ELAVIA II, and GENELAVIA HIV 1+2 (Sanofi-Diagnostics Pasteur, France); and ETI HTLV-III (Sorin Biomedica S.p.A., Italy). All these assays were performed and validated according to the technical specifications of the manufacturers. The discrepant results, seroconversions, and positive samples were confirmed with a WB assay (New LAV blot I and New LAV blot II; Sanofi-Diagnostics Pasteur). The interpretation of the WB results was done according to World Health Organization criteria (19).

RESULTS

Study of positive and negative samples at random. A total of 948 positive samples were studied (Table 1): 704 anti-HIV-1 positive samples, 222 anti-HIV-2 positive samples, 8 double-reactive samples, and 14 low-positive anti-HIV-1 samples (BBI panel); 1,699 negative random fresh samples from a low-risk population (blood donors) were used. The VIDAS test presented a sensitivity of 100% and a specificity of 99.8%. All positive sera were detected with VIDAS, whether they were anti-HIV-1 or anti-HIV-2 positive or both. Four negative samples (all of them WB negative) were positive with this assay. Furthermore, this test always showed a good discrimination between the signals obtained with negative sera and those obtained with the positive sera (data not shown).

TABLE 3. Sensitivity study with seroconversion samples (BH center)

Seroconversion panel	Date[a] of blood collection	Result[b]				HIV-1 WB results	
		VIDAS HIV 1+2	Behring Enzygnost HIV 1+2	Abbott Recombinant HIV-1 EIA	HIV-1 p24 Ag	Banding pattern	Interpretation[b]
BH5	23.08.88	Pos	Neg	Neg	Pos	No bands	Neg
	06.09.88	Pos	Pos	Pos	Neg	gp160, p24	Indeterm
	23.11.88	Pos	Pos	Pos	Neg	gp160, gp120, p66, p55, p24, p18	Pos
BH10	29.03.90	Pos	Neg	Pos	Neg	p24	Indeterm
	13.04.90	Pos	Pos	Pos	Neg	gp160, p24	Indeterm

[a] Day.month.year.
[b] Interpretation of results, according to World Health Organization criteria (19): Pos, positive; Neg, negative; Indeterm, indeterminate. Results of immunoassays: Pos if signal cutoff >1; Neg if signal cutoff <1.

Specificity study with difficult negative samples. The results of the specificity study are summarized in Table 2. In this study, VIDAS was compared with the Abbott recombinant HIV-1/HIV-2 EIA test. The specificities of the VIDAS assay and the reference test were 98.5 and 97.6%, respectively. Two of the five false-positive samples detected in the VIDAS assay were from the African group, one was from a patient with leprosy, and the other two sera were reactive only to HIV-1 *gag* proteins. Regarding this anti-*gag* group, the cross-reactivity of VIDAS was 3.7% (2 of 54 samples), while the reference test gave 11.1% cross-reactions (6 of 54 samples).

Study of HIV-1 seroconversions. We were able to include in this study two seroconversion panels with a total of five samples (Table 3). In this evaluation, the VIDAS assay showed a good sensitivity, better than those of the reference tests used. The result for sample BH5 (from 23 August 1988) was noteworthy, in that it was positive only for HIV p24 Ag, which was also detected by VIDAS. In addition, we analyzed six samples from incomplete seroconversion panels (panels where we had only the early p24 Ag-positive, WB-negative seroconversion serum); in this group, VIDAS (as well as Abbott recombinant HIV-1 EIA) detected the presence of anti-HIV antibodies in 50% (three of six) of the samples, confirming the good sensitivity of the VIDAS assay (data not shown).

Study of VIDAS interassay variability. To assess the interassay variability, three samples (one negative, one weakly positive, and one strongly positive) were repeatedly tested (13 times for the negative sample, 15 times for the low-titer positive sample, and 12 times for the strongly positive sample) in the VIDAS assay, in the same laboratory (Basurto Hospital, Bilbao, Spain [BHB]), and using the same lot of reagents. The interassay variability was 23% (mean RFV signal = 25.07) for the negative sample, 3.97% (mean RFV signal = 507.6) for the weakly positive sample, and 3.88% (mean RFV signal = 1,878.2) for the strongly positive sample. Apparently the variability for the negative sample was very high; however, because of the very low VIDAS signal detected for this sample, the variation was only 25.07 ± 5.8, which is very far from the result obtained for the weakly positive sample (mean = 507.6 ± 20.15).

Comparative performances with other ELISAs. We compared the sensitivity and specificity of VIDAS with those of other ELISAs. The results are summarized in Table 4. All the tests showed an excellent sensitivity (100%), except for the

TABLE 4. Comparative evaluation of sensitivities and specificities of VIDAS HIV 1+2 and several ELISAs

Evaluation center and assay	Source of antigens	No. of samples[a]		% Specificity	No. of samples[a]		% Sensitivity
		Negative	False positive		Positive	False negative	
SUC							
VIDAS HIV 1+2	SP	164	1	99.5	184	0	100
ETI HTLV-III	VL	164	18	90.3	184	0	100
Enzygnost anti-HIV micro	VL	164	0	100	184	3	98.4
BHB							
VIDAS HIV 1+2	SP	171[b]	0	100	133	0	100
Recombinant HIV-1 EIA	RP	177[b]	4	97.7	133	0	100
FP							
VIDAS HIV 1+2	SP	249	0	100	204	0	100
ELAVIA HIV I and HIV II	VL	249	—	—	204	0	100
BH							
VIDAS HIV 1+2	SP	104	0	100	380	0	100
Recombinant HIV-1 EIA	RP	104	—	—	380	0	100
RBTC							
VIDAS HIV 1+2	SP	1,081	3	99.7	IN	—	—
GENELAVIA MIXT	SP	1,081	3	99.7	IN	—	—
DH							
VIDAS HIV 1+2	SP	262[c]	5	98.1	IN	—	—
Recombinant HIV-1/HIV-2 EIA	RP	262[c]	4	98.5	IN	—	—

[a] IN, insufficient number of samples; —, not done.
[b] Including 41% (70 of 171) of difficult negative samples.
[c] Including only difficult negative samples.

Behring assay in the Specialized University for Catholics (SUC) center, for which the sensitivity was 98.4%. Regarding the specificity results, they were very similar for all the tests except for the VL-based SORIN assay (SUC center), with a specificity of only 90.3%. The specificity results of both VIDAS and Abbott tests in the Dupuytren Hospital (DH) center were noteworthy, since in this place all the samples analyzed were from the difficult-negative group. In the Regional Blood Transfusion Center (RBTC), we were able to test VIDAS specificity with fresh negative samples at random. For both VIDAS and SP-based GENELAVIA tests, the specificity was equal to 99.7%.

DISCUSSION

Although SP- and RP-based ELISAs have been used for screening human sera for the presence of anti-HIV antibodies, there has been some concern whether SP- and RP-based tests are of sufficient sensitivity to detect all anti-HIV-positive samples (5, 13). To determine the sensitivity and specificity of a new automated SP-based ELISA system (VIDAS), we performed a European multicenter evaluation in which we were able to compare the sensitivity and specificity of VIDAS with those of other SP, RP, and VL ELISA systems. For this evaluation we selected a wide range of HIV-1- and HIV-2-positive samples and, particularly, negative samples of different origins and in some cases with difficult interpretation because of the presence of immunoglobulins directed against antigens that sometimes cross-react with HIV. We also compared the VIDAS assay with several ELISAs from different manufacturers with different antigen sources (recombinant proteins, synthetic peptides, or viral lysate).

The VIDAS assay showed a good sensitivity (100%) with both HIV-1- and HIV-2-positive samples. With some sera the test was even superior to the reference tests, particularly for seroconversion samples, where it was more sensitive than the other ELISAs and WB tests. In fact, VIDAS detected the first serum specimen from the seroconversion BH5, analyzed in the Bretonneau Hospital (BH) laboratory, that was positive only for HIV p24 Ag. This is an extremely important advantage for detection of infected people prior to seroconversion, particularly in the screening of blood donors. Despite the very limited number of complete seroconversion panels to prove this observation, we have confirmed the sensitivity of the VIDAS test by analyzing six samples from incomplete seroconversion panels (early seroconversion samples that were p24 Ag positive and WB negative), and we were able to detect anti-HIV antibodies in 50% (three of six) of them (data not shown). The overall specificity of the VIDAS assay was 99.6% (2,022 of 2,031 samples), including 16.3% difficult-negative samples (332 of 2,031 samples), such as rheumatoid factor-positive sera, anti-*gag*-positive sera, and African sera. These potential false-positive specimens are usually associated with a reduced specificity observed in other HIV-antibody screening tests, particularly in those based on VL ELISA procedures (7, 9). Nevertheless, as shown in Table 2, both the VIDAS assay and the reference test are very specific regarding these difficult negative sera. With fresh negative samples selected at random, the specificity was 99.8% (1,695 of 1,699 samples). The meaning of the four false-positive results is not clear. Since VIDAS sensitivity is very high, we can speculate that they represent early seroconversion samples that were already detected by the VIDAS test. Unfortunately, we cannot confirm this hypothesis, since sequential serum samples were not available for testing.

In conclusion, on the basis of this evaluation, VIDAS assay showed a very good performance in terms of sensitivity and specificity, confirming the results obtained by others in a smaller evaluation (18). In addition, it shows an increased specificity and, in some cases, sensitivity compared with those of other ELISAs. Furthermore, the results are obtained in 32 min, without any handling after dispensing the samples. This is a very important advantage in order to minimize the risk of human error and therefore to improve the quality of results. These features make this assay an excellent alternative to the long incubations and frequent manipulations, usual in other conventional ELISA tests.

ACKNOWLEDGMENTS

We thank E. Suiphon and N. Phillipe for technical assistance and helpful discussions.

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